MICROBIOLOGICAL REVIEWS, Sept. 1988, p. 327–336
0146-0749/88/030327-10$02.00/0
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Vol. 52, No. 3

Genetic Mechanisms of Bacterial Antigenic Variation

H. STEVEN SEIFERT[1]* AND MAGDALENE SO[2]

Department of Microbiology and Immunology, Northwestern Medical and Dental Schools, Chicago, Illinois 60611,[1] and Department of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California 92037[2]

INTRODUCTION

The ability of an organism to respond and adapt to the environment is the keystone to its survival as a species. Pathogenic microorganisms have evolved a variety of mechanisms to deal with the rigors of the host immune response. These mechanisms, depending on the life cycle of the pathogen, include mimicry of host antigens, survival within professional phagocytes, and antigenic variation of major surface antigens (see reference 7). Not surprisingly, many pathogenic microbes which are constantly exposed to antibodies within the host have evolved the latter mechanism to deal with the immune response. The purpose of this article is to discuss in detail the genetic mechanisms of bacterial antigenic variation. For a review on antigenic variation of the African trypanosomes, we refer the reader to Borst and Cross (8).

Broadly defined, antigenic variation refers to the ability of a microbe to alter the antigenic character of its surface components. The ability of a microbe to undergo antigenic variation is defined here as the ability of a single strain to express several antigenic variants of a cellular component, with the rate of change of the component being significantly higher than the mutation rate. The simplest form of antigenic variation involves a biphasic transition resulting from a rapid on-and-off switching of gene expression modulated by recombination at the deoxyribonucleic acid (DNA) level. Such switches occur for the type 1 pili of *Escherichia coli* (1), pilin of *Neisseria gonorrhoeae* (66, 69), and protein II (P.II) of *N. gonorrhoeae* (75). Some bacteria alternately express only two antigenic types of one protein. *Salmonella typhimurium*, the best-studied example, alternately expresses two flagellin genes, H1 and H2. Differential expression of H1 and H2 (also known as the flagellar phase transition) is controlled at the DNA level, involving an invertible segment of DNA, a process which ultimately allows the expression of one flagellin gene while preventing the expression of the other (68, 94). DNA inversion events also control the alternate expression of genes encoding pilins of *Moraxella bovis* (48b), as well as genes encoding the tail fiber components of bacteriophages Mu and P1 (see reference 58).

All bacterial species show interstrain variation of surface determinants. This is largely due to antigenic drift or the slow accumulation of mutations in the structural genes encoding the surface components. Examples of systems which show antigenic drift are the streptococcal M protein (20, 45) and the lipopolysaccharide of *Salmonella* spp. (55). Antigenic drift is postulated also to contribute to the emergence of hemagglutinin and neuraminidase variants of the flu virus (see reference 93). Viral pathogens also show antigenic shift, the exchange of major antigenic determinants between strains in mixed infections. Antigenic shift is analogous to bacterial antigenic variation as defined here, as the former is believed to result from recombination between partially homologous DNA sequences in mixed infections. The two processes differ in that antigenic variation occurs during any infection with a single strain, while antigenic shift requires a mixed infection with two variant strains.

In contrast, some bacterial pathogens can express in succession numerous variants of a protein or other surface component which are distinguishable from each other serologically. The most intensely studied systems are the pilin and P.II proteins of *N. gonorrhoeae* and the variable major protein (VMP) of *Borrelia hermsii*. In these cases, a single cell has the genetic information necessary for production of a large number of each of these variant proteins. In *N. gonorrhoeae*, variation of both pilin and P.II proteins have been observed in vitro and in vivo.

Thus far, we have discussed antigenic variation in the context of immune surveillance. It is often assumed that pathogenic microbes have evolved antigenic variation for the

* Corresponding author.

sole purpose of evading the host immune response. However, we must keep in mind that antigenic variation of surface components could also serve other purposes, such as adaptation of a microbe to various environments. This is most clearly demonstrated by the i-antigen system of *Paramecium aurelia* (see below). Pathogens may also use antigenic variation to modulate their host range. *N. gonorrhoeae* strains producing gamma or delta pilins apparently differ in their binding affinities for different epithelial cell types (44). Variant *papG* proteins on the pili of *E. coli* urinary tract isolates attach to different cell types (48a; D. Low, personal communication; see also below).

MECHANISMS OF ANTIGENIC VARIATION

N. gonorrhoeae Pilin

Pilus function. The pilus is an outer membrane organelle composed of repeating subunits of the 18 to 24-kilodalton pilin protein and possibly several other proteins in minor amounts. It facilitates adherence of the bacterium to a variety of eucaryotic cell types (10, 33, 79, 92), and is thought to play a role in bacterial interaction with neutrophils (34, 54, 61, 87, 88). Piliated gonococci are naturally competent for DNA transformation (73), although transformation with *Neisseria* DNA occurs preferentially (17). Piliated cells will transform chromosomal markers at a frequency 3 to 4 logs higher than their nonpiliated counterparts (73), although the pilus component involved in these interactions has not been identified.

Evidence for pilin antigenic variation. Results of early studies suggested that pilin undergoes extensive antigenic variation. Pilins from different clinical isolates vary in size, isoelectric point, and amino acid composition (41). They also differ in their binding affinities for epithelial cells (44) and are immunologically distinct (11). Antisera raised against pili from one isolate will cross-react best with homologous pili, but only minimally with pili from other strains. Not surprisingly, pili of one strain protect human volunteers against challenge only by the homologous strain (9). Partial amino acid sequences obtained for pilins from two clinical isolates indicate that the pilins share extensive homology within the range of residues determined (approximately 23 residues [27]).

In vitro studies have shown that a single cell of one gonococcal strain is capable of giving rise to many progeny which now produce pilins with new epitopes (24, 52). A comparison of expressed variant pilin sequences from these strains indicated that the pilin gene can be divided into constant (C), semivariable (SV), and hypervariable (HV) regions (Fig. 1a). The C region encodes approximately the first 50 residues of each mature pilin and is invariant in sequence in all cases examined. The variant pilin sequences differ from each other to a small degree in the central, or SV, portion of the gene. This region is characterized mainly by single base changes which result in single amino acid changes of the protein. In the last third of the pilin gene (the HV region) are found insertions and deletions of one or more codons in multiple sites, as well as single codon changes. Epitope mapping studies with pilin-specific monoclonal antibodies indicate that this region encodes the most antigenic portion of pilin (52). Although the latter two-thirds of the pilin gene is considered variable, it does contain small invariant regions. The two most striking of these have been termed *cys-1* and *cys-2*, after the cysteine residue each encodes, which flank the HV region. Presumably, the invariant amino acids in pilin are critical to the maintenance of pilus structure and perhaps to other functions as well.

Two approaches have been used to examine the arrangement of pilin sequences in the gonococcal chromosome. DNA sequencing of *pilS1*, a major pilin-hybridizing locus near the two expression sites, showed that this region of about 3 kilobase pairs contains six variant pilin sequences oriented in the same direction as the expressed gene(s) (23) (Fig. 1c). Each gene copy contains the entire variable region up to, but not always including, the termination codon of pilin. Each also contains some constant 5′ sequences, though the 5′-endpoints differ from one gene to the other. No pilin promoter sequences are found in the locus. A comparison of the *pil* variant sequences in *pilS1* shows that these partial genes can also be divided into C, SV, and HV regions. The comparison of silent sequences allowed Haas and Meyer (23) to propose that minicassettes of variable pilin sequences found in silent pilin loci are introduced as discrete units into an intact variant pilin gene in the expression site. One such minicassette sequence (from copy 1 or 3) from a major silent *pil* locus, *pilS1*, has been observed to have donated its sequence to the expression site during pilin antigenic variation. Others have also shown recombination of other partial *pilS1* gene copies (from copy 5) into an expression locus (85). The conserved sequences within the pilin structural gene are probably used as crossover points for the recombination reactions and are most likely required to maintain the structural integrity of the pilus.

The arrangement of pilin sequences in the gonococcal chromosome also has been examined by Southern hybridization, using synthetic oligonucleotide probes specific for several regions of two variant pilin genes. These studies (67) essentially support the DNA sequence findings described above and further identify other silent *pil* loci whose variant sequences are used by the cell to produce antigenically variant pilins. The above studies have identified seven silent *pil* loci (termed *pilS1–7*), although there may be additional undiscovered silent loci. It is now accepted that pilin antigenic variation results from the replacement of the expressed pilin sequence in an expression locus by part or all of a variant sequence from a silent copy. The requirement for the gonococcal *recA* homolog in promoting the recombination reactions between the silent and expressed genes (38–40) suggests that the exchange involves the formation of heteroduplex DNA.

In addition to copies of variant *pil* genes, the silent loci share other sequence homologies with the expression sites *pilE1* and *pilE2*. Of note is the *Sma/Cla* repeat, which is present at the 3′-untranscribed region of the complete pilin sequence in each expression site, as well as in each silent locus (23, 56b, 67; T. F. Meyer, personal communication) (Fig. 1). All loci that contain pilin sequences also contain the *Sma/Cla* repeat. Other DNA repeats have also been identified in silent and expressed pilin loci. These include RS1, RS2, and RS3 (23, 56b) (Fig. 1c). The function of these repeats has not been determined. However, it is probable that some or all of them will be involved in the recombinational process which leads to pilin antigenic variation.

Two models of pilin antigenic variation. Southern hybridization studies based on oligonucleotide probes have given rise to one model of pilin antigenic variation. Each HV-specific oligonucleotide hybridizes to one silent locus in all derivatives of strain MS11. In those progeny which now actively express the variant pilin gene from which this HV sequence is derived, the probe also hybridizes to the pilin expression locus. Such data (23, 66, 84) indicate that pilin

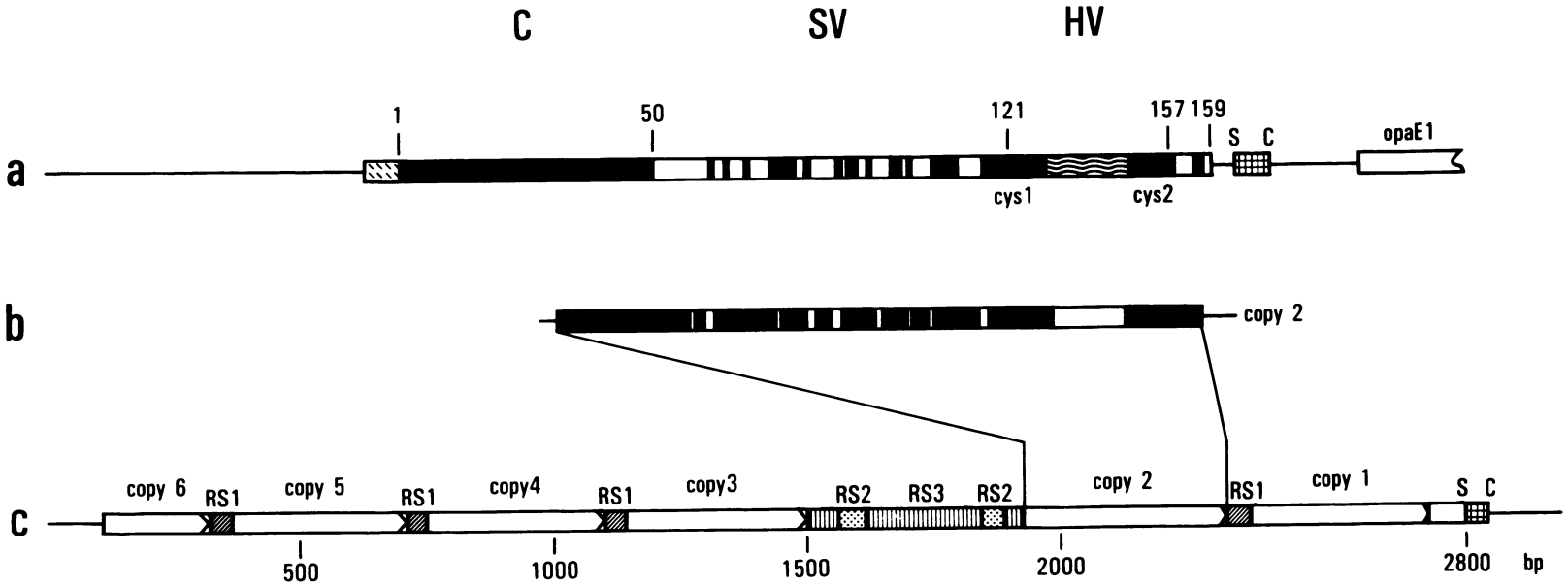

FIG. 1. Gonococcal pilin loci. (a) Map of pilin structural gene: C, constant region; SV, semivariable region; HV, hypervariable region. The numbers correspond to amino acid residues in the mature protein. Black boxes are residues conserved between the original MS11 protein (51) and the MS11 variants developed by Bergstrom et al. (6) and the P9 variants of Nicolson et al. (52). Open boxes indicate regions where single amino acid substitutions occur, and wavy lines show region where multiple insertions and deletions occur. Diagonal dashed box represents the seven-amino-acid leader sequence that is conserved among all pilins. *cys1* and *cys2* indicate the conserved cysteine residues at positions 121 and 157, respectively. The checked box bordered by S and C indicates the location of the *Sma/Cla* repeat in the DNA sequences surrounding the structural gene. The open box downstream of this repeat shows the location of one P.II gene sequences, *opaE1*. (b) Comparison of amino acid changes between *pilS1* copy 2 and the original MS11 sequence. Black boxes show conserved residues, while open boxes indicate variable regions. (c) Map of pilin sequences in *pilS1*. Locations of truncated pilin gene sequences (copies 1 to 6) and repeat units (SC, RS1, RS2, and RS3) taken from Haas and Meyer (23), bp, Base pairs.

antigenic variation results when a copy of a variant pilin gene in a silent *pil* locus is recombined into an expression site. However, the pilin sequence displaced from the expression site during recombination is not found in the silent locus which has actively donated its variant *pil* gene, nor is it found anywhere else outside its own silent locus. These results suggest that recombination between the silent and expression loci is a nonreciprocal event. This unidirectional transfer of genetic material within a cell would be classified as gene conversion.

Gene conversion occurs in a number of eucaryotes such as yeasts (29, 30, 36, 37), African trypanosomes (46), and mammalian cells (18, 64, 69). However, this reaction occurs at a low frequency in procaryotes. In *S. typhimurium*, gene conversion occurs at a frequency of approximately 10^{-6} (J. Roth, personal communication). If pilin-related gene conversion events in the gonococcus were to occur by general recombination, they may be expected to occur at a frequency similar to that of *S. typhimurium*. Such a frequency is lower than that observed for pilin variation. Therefore, if gene conversion is responsible for pilin recombination, it would have to be a specialized system which gives a high rate of recombination, but which only allows for nonreciprocal recombination. Such a system has been described for mating type interconversion in *Saccharomyces cerevisiae* (see reference 28).

Two aspects of the gonococcal life cycle lend support to an alternate model of pilin antigenic variation. As mentioned earlier, the gonococcus is naturally competent for DNA transformation (73) and has a strong preference for its own DNA (17). For piliated cells the frequency of transformation of chromosomal markers such as antibiotic resistance and auxotrophic markers is approximately 10^{-2} to 10^{-3}. The transformation frequency for nonpiliated cells is 3 to 4 logs lower (73). The gonococcus also undergoes autolysis readily (25). In an actively growing culture, a certain percentage of cells have already lysed. Thus, living gonococci are constantly exposed to free DNA, and transformation of chromosomal markers between strains in mixed culture has been reported (63). These observations suggest that the gonococcus has evolved the process of DNA transformation as a specific rapid adaptive response, rather than as a long-term mechanism to acquire new traits for the species in general. Thus, DNA transformation very well could be used by gonococci to undergo pilin antigenic variation. According to the DNA transformation model, antigenic variation begins when DNA from a lysed cell is taken up by a living cell. The gonococcal chromosome contains many silent variant *pil* genes. If the incoming DNA contains a silent *pil* locus, it would preferentially recombine at a site with which it shares homology, such as the *pil* gene in the expression site. The product of such a recombination event would be a variant cell that now expresses a new pilin gene. Figure 2 is a diagrammatic representation of both models of pilin antigenic variation.

To examine pilin-related recombination events, an assay has been developed to allow quantitation of recombination frequencies between pilin loci in the gonococcus (H. S. Seifert, R. A. Ajioka, C. Marchal, P. F. Sparling, and M. So, submitted for publication). An operon fusion was created, in the gonococcus, in which the chloramphenicol acetyltransferase structural gene (the CAT cartridge; 13) was inserted downstream of the pilin promoter sequences and upstream of the intact pilin structural gene in *pilE1* (Fig. 3, strain MS11CAT8). Translation of each gene is regulated by its own ribosomebinding site, and transcription of both genes is

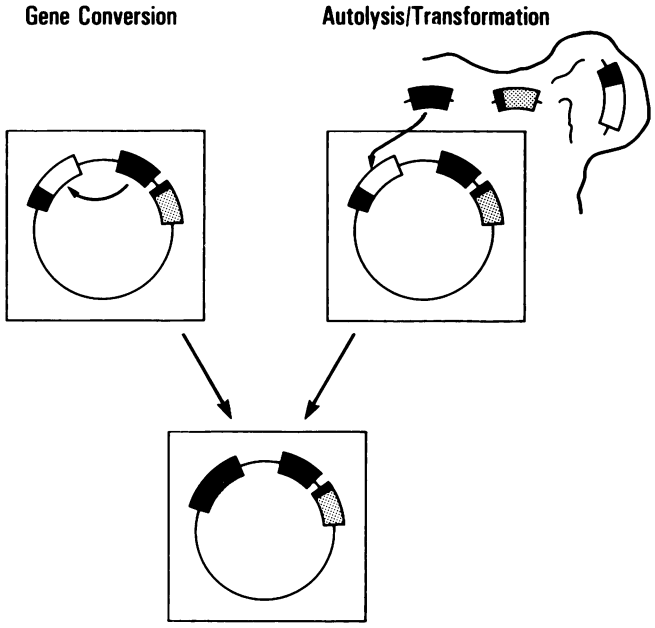

FIG. 2. Diagrammatic representation of extracellular versus intercellular models of pilin antigenic variation. Open box represents the pilin gene being expressed by the gonococcal cell. Hatched and stippled boxes represent variant pilin sequences in the gonococcal chromosome. The cell on the left is switching its pilin variant by an intracellular gene conversion mechanism. The cell on the right is switching its pilin gene sequence by uptake and recombination with free DNA released from a neighboring cell that has autolyzed. For simplicity of representing the models, the gonococcal cells are not shown as diplococci.

controlled by the pilin promoter. The other expression locus expresses a wild-type pilin gene. Nonreciprocal recombination events involving pilin loci can be scored by plating cells on chloramphenicol levels that select against those with one copy of CAT, but allow the growth of those with two copies of CAT. The rate at which the wild-type expression site receives the CAT gene from the other site would reflect the frequency of gene conversion, and this approach can be used to quantitate such events.

To test the hypothesis that DNA transformation is the basis of pilin antigenic variation, the frequency of the above "gene conversion" event was measured in a strain deficient in the DNA uptake step of transformation. The DNA uptake mutation from strain FA660 (*dud-1*; G. Biswas and P. F.

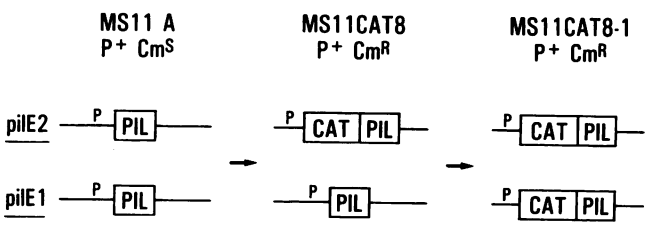

FIG. 3. Model for pilin gene conversion. A diagram showing the structure of pilin expression loci, *pilE1* and *pilE2*, in three strains, MS11-A, MS11CAT8, and MS11CAT8-1. PIL indicates pilin structural gene, while CAT represents the chloramphenicol acetyltransferase structural gene. The uppercase P indicates the location of the pilin promoter relative to the structural genes. P^+ denotes piliated phenotype and Cm^S denotes sensitivity to chloramphenicol, while Cm^R denotes resistance to chloramphenicol.

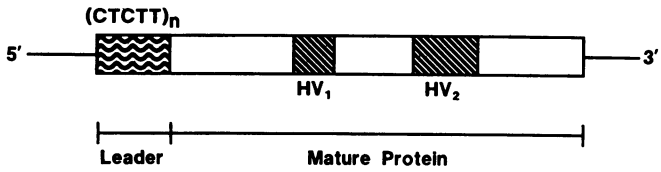

FIG. 4. Map of P.II structural genes. Wavy lines represent the membrane transport leader sequence containing the CTCTT repeat. Open boxes denote conserved regions of the protein. Hatched boxes indicate the variable regions, HV1 and HV2.

Sparling, personal communication) was transferred to MS11CAT8 by transformation, and gene conversion frequencies between expression sites in the wild type and the *dud-1* mutant were compared. The transformation-proficient parent strain gave at last 100-fold-higher levels of progeny colonies with two CAT genes than the *dud-1* strain. These results do not rule out the possibility that gene conversion occurs in the gonococcus or that gene conversion plays a role in pilin antigenic variation. However, the data indicate that DNA transformation is a major mechanism used by gonococci to regulate interaction between pilin loci. Figure 2 shows the difference between these two models for antigenic variation.

The transformation model for pilin antigenic variation is consistent with all of the published data supporting the gene conversion model for antigenic variation. The major difference is that the gene conversion model allows a single bacterial cell to undergo antigenic variation, while the transformation model requires the colonizing bacterial population to contribute to the survival of the population via lysis of a few individual cells.

The DNA transformation model of pilin antigenic variation is attractive for several reasons. DNA transformation and autolysis occur at a high enough frequency to allow high-frequency pilin antigenic variation to occur. In this model, all silent *pil* loci should have an equal probability of donating their sequences to the expression site; there is no bias for certain sequences due to their chromosomal location. Finally, all *pil* loci could donate their sequences to all other *pil* loci. Recombination between two silent *pil* loci could, in some cases, create a new variant *pil* gene and thus increase the repertoire of *pil* genes within a population of cells.

N. gonorrhoeae P.II

P.II function. Another major gonococcal surface protein which undergoes phase and antigenic variation is the ~30-kilodalton opacity protein, or P.II. P.II promotes adherence of gonococci to each other (43, 91), adherence to host eucaryotic cells (43), and resistance of bactericidal effects of normal human serum (32, 43). In vitro, the number of variant P.II proteins produced by a single strain ranges from none to several (42, 80, 91). P.IIs produced by a single strain differ in both molecular weight and immunological cross-reactivity (43, 82, 83). The similarity of peptide maps of variant P.IIs suggests that the proteins share common domains (26, 81). Not surprisingly, surface-exposed regions of P.IIs show the greatest degree of antigenic differences, while those regions buried in the bacterial membrane show the least (83). The menstrual cycle seems to affect the degree of P.II expression (31), and P.II-expressing variants were isolated from volunteers inoculated with a P.II-nonexpressing strain (86).

P.II genetics. Southern hybridization studies with a cloned P.II gene probe strongly suggest that the gonococcal chromosome contains many copies of P.II genes (77). However, differential expression of the P.II gene does not appear to be accompanied by genome rearrangement (75, 77), in contrast to the pilin expression system (see above). The same studies also showed that at least one P.II gene is closely linked to *pilE1*, an expression site for pilin (77).

DNA sequence analysis of two cloned P.II genes expressed successively by a single strain showed that both genes were derived from a single locus closely linked to *pilE1* (75). These data suggest that individual P.II genes vary their antigenic properties by DNA recombination. The studies also confirmed earlier observations (26, 81) that variant P.II proteins share common domains. Sequence data from several cloned P.II variant genes has shown that the variable region is concentrated in two areas of the protein, HV1 and HV2 (15a, 75) (Fig. 4).

The promoter regions of the P.II genes derived from this locus (*opaE1*) contain the standard −35 and −10 sequences. A ribosome-binding sequence was also present approximately 50 base pairs downstream of the −10 sequence. The open reading frame of these two P.II genes were located by comparison of the partial amino acid sequence with that deduced from the DNA sequences. A series of eight repeats of the sequence (CTCTT) immediately precedes the codon for the N-terminal alanine of the mature protein (Fig. 5). This repetitive pentameric sequence is itself preceded by an ATG start codon. Analysis of the codons in frame with this ATG indicates that the N-terminal portion of the translational product has the length and the hydrophobicity profiles of a "classical" signal sequence. Since P.II is an outer membrane protein and therefore requires translocation across the bacterial membrane via a signal sequence, the region encoded by the CTCTT repeats was proposed to form part of the P.II signal sequence.

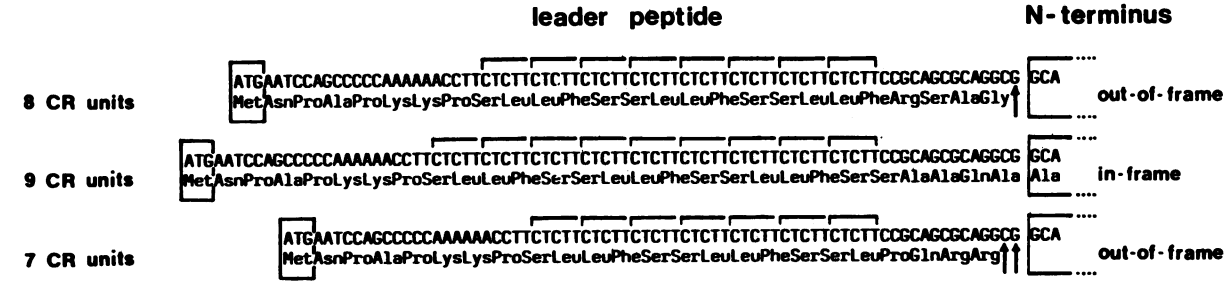

FIG. 5. Alteration of P.II protein reading frame. This figure is reprinted from Stern et al. (75) with permission of the authors and *Cell*. The expansion and deletion of CTCTT repeats alter the relationship between the initiating AUG codon and the codons for the mature P.II protein.

Southern blots of chromosomal DNA, using a synthetic oligonucleotide containing the CTCTT repeat, showed that this sequence is associated with many if not all P.II genes (75). Other P.II genes were isolated from a gene bank derived from one P.II-expressing strain, using the CTCTT oligonucleotide as probe. Mapping studies showed that these clones were derived from different regions of the chromosome. In addition, approximately 25% of the clones also produced apparently native sized P.II proteins in *E. coli*, indicating that the cloned P.II genes contain the coding sequence for the entire protein. Thus, in contrast to the *pil* system, each P.II gene is a potential P.II expression site.

Primer extension sequence determination of the CTCTT region of P.II messenger ribonucleic acids from several derivatives of one gonococcal strain indicates that the CTCTT repeats are transcribed and thus are truly part of the coding sequence of the P.II gene. Furthermore, most, if not all, P.II genes are transcribed in all of the derivatives whether or not the cells produced P.II proteins. However, the number of pentameric CTCTT units present in these transcripts ranged from 8 to 13. A transcript containing the correct number of CTCTT repeats, in relation to the ATG start codon, would contain the correct P.II reading frame (Fig. 5). DNA sequence analysis of several cloned P.II genes also showed that they contain different numbers of the CTCTT unit (15a).

Models for P.II regulation. These data support a model of P.II phase and antigenic variation which involves translational control coupled with DNA recombination. Several regions of the gonococcal chromosome contain intact P.II genes, and all P.II genes are transcribed within a cell at any given moment. The CTCTT repeats at the 5′ end of the gene encode the signal sequence of the P.II protein. The correct number of pentameric repeats in a gene would place codons of the signal sequence in frame with those of the mature protein. The translational product of such a gene would be a full-length variant P.II protein. Genes with the incorrect number of CTCTT repeats would encode a truncated P.II product. If none of the genes contain pentameric repeats in frame with the codons for the mature protein, the cell would not make P.II, and a P.II$^-$ phenotype is the result. A single P.II gene could be turned on and off by a yet to be defined process which varies the number of its CTCTT repeats.

In bacteria, tandemly repeated sequences are subject to recombination, a process which may result in deletion or expansion of the number of repeats. A P.II gene with the correct number of pentameric repeats could, after recombination with another gene or a copy of itself, become one containing an incorrect number of repeats. Such a recombination event could be *recA* mediated, but the switch from transparent to opaque colony morphology has been reported to be independent of *recA* functionality (38), although relative frequencies of the switch were not reported. Therefore, *recA* independent recombination between pentameric repeats, or another mechanism such as replication fork slippage, may form the basis of the on/off switch for P.II genes. Furthermore, recombination between two variant P.II genes would lead to the generation of new variant sequences. This even has been observed in one such P.II locus, *opaE1* (15a, 75; C. Marchal, personal communication). One can imagine that DNA transformation would play a role in P.II gene expression as well. It could contribute to the generation of new P.II variant genes, as occurs with pilin antigenic variation, and may also affect the CTCTT region of these genes. The relative contribution of these processes to P.II variation has not been reported.

Borrelia VMP Variation

For years, the borrelias have been cited in medical microbiology courses as the primary example of bacterial antigenic variation. Members of this genus cause relapsing fever, a clinical syndrome characterized by successive bouts of fever in humans and other animals (see reference 19). Infection of the host, via an insect vector, often leads to persistent bacteremia. The febrile crises are brought about by the presence of large numbers of the spirochetes in the blood stream. Resolution of the fevers is accompanied by the appearance of antibodies specific for the spirochete responsible for the preceding attack (14, 16, 50). The spirochetes which produce the next bout of fever will induce their own specific antibodies. Upon injection of a rat with a single spirochete, antigenically distinct spirochetes can be isolated from the blood of the animal during the relapse phases (65). The characteristics of this disease suggest that surface antigens of the spirochetes undergo antigenic variation. Thus, growth of spirochetes of one predominant type is responsible for a relapse. The resolution of the relapse is due to the clearance from the blood of spirochetes by antibodies specific for that spirochete population. The appearance of the next relapse would be due to the outgrowth from this population of new variant spirochetes.

VMP antigenic variation. Details of borrelia antigenic variation have been studied in *B. hermsii*, a genus which causes relapsing fever in mice. Infection studies showed that a single organism can give rise to at least 24 new serotypes (78). Thus, the bacterium contains many copies of variant genes that encode the cellular component which undergoes antigenic variation. The frequency of serotype switching is approximately 10^{-4} to 10^{-3} (78). As is the case in African trypanosomes (8), there is a loose order of appearance of the serotypes in the host, and certain serotypes predominate in the early stages of the disease (78). Serotypes cleared from one host can reappear upon passage of the spirochete to a second host (15). This finding indicates that genetic information for serotype specificity is not lost during antigenic variation, a necessary condition for a successful antigenic variation system.

The appearance of new serotypes of *B. hermsii* is correlated with a change in a major protein of approximately 40 kilodaltons (4). The antigen has been named the VMP. Surface iodination (4) and antibody agglutination studies indicate that the VMP is a surface-exposed membrane protein. VMPs of serotypic variants derived from a single organism of *B. hermsii* HS1 differ in molecular weight, peptide map, and reactivity with serotype-specific antisera, both polyclonal and monoclonal (3, 4). Partial protein sequence data obtained for VMP_7 and VMP_{21} indicate that serotype specificity is due to differences in primary structure of the VMPs, although there is significant amino acid sequence homology between the proteins (5).

Genetics of VMP antigenic variation. Differential expression of the VMP genes is accompanied by DNA rearrangements (49). A VMP-specific oligonucleotide probe hybridizes to a restriction fragment common to all isogenic variants of one *B. hermsii* strain, differing only in the variant VMP produced. In the strains that express the oligonucleotide-specific VMP, an additional fragment can be detected. These data strongly suggest that each VMP gene exists as a silent copy in the borrelial genome and that expression of a VMP gene is the result of duplication and placement of this VMP sequence into an expression site. The DNA sequences 5′ of expressed VMP genes are identical. However, the 5′-

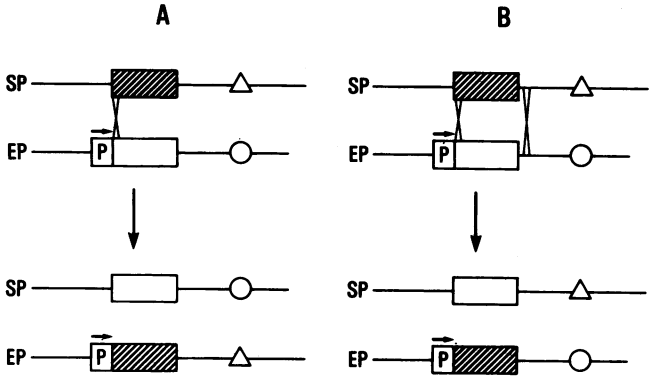

FIG. 6. Models for *Borrelia* VMP variation. (A) Single site-specific recombination reaction that replaces all of the sequences 3′ to the expressed VMP gene in the expression plasmid (EP) with those that are 3′ to the new VMP gene in the storage plasmid (SP). (B) Double site-specific recombination reaction that replaces the sequences immediately 3′ to the expressed VMP gene with the sequences immediately 3′ to the new VMP gene from the SP, but with another undefined recombination site occurring prior to the end of the SP and EP. The triangle and circle represent undefined genetic elements within the SP and EP 3′ to the expression site. The uppercase P represents the promoter of the EP, with the arrow indicating the direction of transcription.

flanking regions of unexpressed equivalents do not share any homology with each other (57). These data indicate that expression of a VMP gene results from the juxtaposition of that particular VMP structural gene with an expression site. These biological features are reminiscent of trypanosome variable surface glycoprotein antigenic variation and *N. gonorrhoeae* pilin antigenic variation.

The genes encoding VMP_7 and VMP_{21} have been cloned into *E. coli* (49). Studies with orthogonal gel electrophoresis and Southern blots with the cloned genes as probes indicate that the VMP genes are located on multicopied linear plasmids (57). Furthermore, the ends of the plasmids appear to be modified. Recent data suggest that the ends of the linear plasmids are covalently closed (A. Barbour, personal communication).

Models of VMP antigenic variation. Figure 6 shows the current model proposed for VMP antigenic variation. VMP genes are stored on linear plasmids as silent information. They are not transcribed, presumably because of the lack of promoter information. Each silent gene is apparently a complete copy of the structural gene. When recombination takes place between a storage plasmid and an expression plasmid at the VMP locus, the VMP gene now present in the expression site is activated, resulting in a cell which makes a new VMP.

These data suggest that VMP antigenic variation occurs by a site-specific recombination reaction between a storage plasmid and the expression plasmid which places the new VMP gene sequence downstream of the expression signals in the expression plasmid. It appears that part or all of the information 3′ of the new gene is also brought along with the expressed gene. It is not clear whether the recombination reaction is a single site-specific recombination reaction that places the new VMP gene downstream of the expression signals along with all of the 3′ plasmid sequences or there is a second recombination site 3′ to the new VMP gene that also undergoes recombination, leaving the original end on the expression plasmid. Both of these models are represented in Fig. 6. Because the plasmids with VMP information are multicopied in nature, a VMP recombination event between one storage and one expression plasmid should result initially in a cell which contains copies of both expression plasmids and therefore makes both VMPs. The outgrowth of cells which express predominantly one VMP serotype could be the result of plasmid segregation or active removal of the previous expression plasmid, aided by selection forces exerted by the host antibody response. As mentioned earlier, the switching frequency is quite high (10^{-4} to 10^{-3}). Assuming that the VMP-associated recombination is random, a fairly dense population of *B. hermsii* would be predicted to contain more than one VMP serotype. Such a mixed population of serotypes has been observed in animal infection experiments (78).

OTHER SYSTEMS THAT MAY UNDERGO ANTIGENIC VARIATION

This review has covered in detail three procaryotic systems which exhibit antigenic variation. Other bacteria also vary their surface coats, although the mechanisms involved are not understood as well. *N. meningitidis*, a close relative of *N. gonorrhoeae*, is a major cause of bacterial meningitis. The meningicoccus produces opacity$^-$ (or P.II$^-$)-like proteins which also exhibit intrastrain differences (59, 60). A recent study highly suggests that P.II variation in the meningicoccus uses a mechanism similar to that which effects P.II expression in the gonococcus (76). The meningicoccal chromosome contains many P.II genes. Each gene is transcribed, and each contains a variable number of the CTCTT repeat. Thus, differential expression of the meningicoccal P.II-like proteins is most likely also due to the variability of the number of CTCTT repeats.

N. meningitidis also produces pilin similar to that of *N. gonorrhoeae* (27, 74). Interstrain differences of meningicoccal pilin have been observed (89), and pilus antigenic variation occurs (56a). The meningicoccus is also highly transformable (12), and a relationship between piliation and transformation competence has been reported (22). *N. meningitidis* also contains silent truncated pilin sequences similar to those of *N. gonorrhoeae* (56c). As mentioned above, the P.II expression systems of both species appear to be regulated by the same mechanism. Given these facts, it is very likely that meningicoccal pilin does undergo antigenic variation and that this variation occurs by the same mechanism(s) defined for gonococcal pilin.

The P-pili of uropathogenic *E. coli* contribute to bacterial colonization of the uroepithelium. It consists of a major pilin subunit (*papA*) and minor proteins located at the tip of the pilus (*papE*, *-F*, and *-G*; 47, 48). Binding of the pilus to the uroepithelium receptor occurs via the *papG* protein (48a). The genes responsible for pilus biogenesis are clustered in the *E. coli* chromosome (53). A survey of urinary tract isolates has identified at least 16 serologically distinct P-pili (56). Recent DNA hybridization studies indicate that the chromosome of some urinary tract infection isolates contain two (B. Lund and D. Low, personal communication) to several (Low, personal communication) copies of the P-gene cluster. One such strain containing two P-pili gene clusters produces two antigenically distinct *papA* pili (Low, personal communication). Other studies indicate that the *papG* gene product from two different clusters have different receptor specificities (48a; Low, personal communication). It is possible that the P pilus of uropathogenic *E. coli* undergoes limited antigenic variation. Studies on receptor-binding specificities suggest that limited antigenic variation may

occur to allow the bacterial pathogen to modulate its host range.

Perhaps the most striking example of the evolution of antigenic variation for purposes other than escape from host immune response is *P. aurelia*. The outer surface of this free-living protozoan consists of a major protein termed the i-antigen (72). Serotyping of *P. aurelia* is based on the antigenic characteristics of this high-molecular-weight protein. A stock of *P. aurelia* is capable of expressing several antigenically distinct i-antigens in succession (71), although i-antigen variation, like the P.II system, does not appear to involve genome rearrangement (21). i-Antigen variation occurs at a rapid rate in response to a variety of environmental signals such as pH, temperature, and food supply (70). The function of the i-antigen is unclear but is thought to be important for the life cycle of the paramecium, as variants lacking the protein have not been obtained.

SUMMARY AND OVERVIEW

The studies described above indicate that procaryotes have evolved a variety of mechanisms to vary their surface coats. *N. gonorrhoeae* primarily uses DNA transformation to effect pilus antigenic variation at the recombinational level. It also uses recombination (and perhaps also DNA transformation) to bring about P.II antigenic variation at the translational level. Finally, *Borrelia* organisms have evolved a plasmid recombination system to undergo VMP antigenic variation.

To place procaryotic antigenic variation into proper perspective, we end this review with a brief consideration of the host immune system. Mammals have also evolved what could be considered an antigenic variation system, i.e., the generation of antibodies with different antigen-binding specificities. The arrangement of multiple copies of V, D, and J gene segments in the mammalian genome is reminiscent of the arrangement of silent pilin gene segments in the gonococcal chromosome. However, unlike pilin, P.II, and VMP expression, the generation of a functional expressing immunoglobulin gene does not involve expression sites. Instead, a complete immunoglobulin gene is created by recombinational joining of various gene segments, with concomitant deletion of intervening sequences. A system that appears to resemble the gonococcal pilin mechanism has been described for chicken immunoglobulin light chains (62). The light chain variants all are derived from a unique V–J rearrangement, with diversification occurring by gene conversion from other V gene copies to this single expressed gene within the Bursa of Fabricius.

Four main processes appear to be responsible for the generation of antibody diversity in mammalian cells (see reference 90). The first, known as "combinational diversity," is the joining of V and J gene segments in various combinations. Diversity could also be generated by imprecise joining at V–J, V–D, and D–J junctions. In addition, joining of the V_H-D and D-J_H segments could lead to insertion of one to several nucleotides at these junctions. Finally, sequence changes could occur in immunoglobulin gene segments by somatic mutation. Whether these four processes also contribute to antigenic variation in procaryotic systems is not known at present. Since both the procaryotic and eucaryotic systems operate at the recombinational level, it is possible that the first three processes which contribute to immunoglobulin diversity also play a role in procaryotic antigenic variation. As for somatic mutations, it is clear that antigenic drift contributes significantly to the generation of hemagglutinin and neuraminidase variants of the flu virus. It is therefore likely that this process also contributes to sequence variability of the pilin, P.II, and VMP genes. In addition, gene conversion is thought to contribute to the generation of somatic mutation in immunoglobin genes (2).

In summary, it is interesting to note that the systems of antigenic variation and immunoglobulin diversification have evolved in a similar and complementary fashion, with DNA recombination playing a central mechanistic role. It is highly likely that the two systems developed together, with each providing the evolutionary pressure needed by the other. Finally, the examples of antigenic variation covered in this review illustrate the fascinating and diverse ways microbes have found to regulate and alter gene expression.

ACKNOWLEDGMENTS

We thank all of our colleagues who communicated results prior to publication, as noted in the text.

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